



Effect of Medicinal plants Extract on Gut Microbial Flora of Broilers and Ammonia Emission in Poultry Litter

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Introduction

The poultry industry is one of the huge and swiftest growing based industries of world. Poultry industry however, due to huge quantity of litter and manure may cause severe environmental issues. The manure comprise enormous amount of urease producing microbes. Urease enzyme break urea and produce ammonia. This ammonia leads to acidification and eutrophication. Ammonia is one of the most toxic gas that cause major problems in broiler shades. Ammonia is responsible for respiratory tracts disease in broilers. Broiler are sensitive to ammonia. Prolong exposure of birds to ammonia may lead to blindness. If the level increase from (50 to 100ppm) may lead to Keratoconjunctivitis. Ammonia is colorless gas, alkaline in nature and irritating smell easily soluble in water, produced from nitrogenous waste of birds in poultry shades due to urease producing bacterial activity. Ammonia also has bacteriosidal activity mean it kill bacteria in poultry litter, but it adversely affects bird's growth rate and other regulatory performance. The word poultry litter is the mixture of wasted feeds, waste material, excretion, bedding material and feather that cover the surface of poultry houses. The bedding material use in poultry shades includes rice hulls, straw, wood shaving, and sand has been also use as bedding material.

Urease producing bacteria cause major problem in poultry shades by producing urease enzyme, which degrade urea into ammonia. When ammonia reacts with moisture than ammonia converted into ammonium (NH₄⁺), which cause respiratory problems in broiler. Gram negative bacteria are mainly responsible in poultry litter to convert uric acid into ammonia but some convert uric acid into urea. The poultry litter contain high amount

of protein, uric acid and microbes. Approximately 52% fiber and mineral and 38% of crude protein. Bacterial urease is the most important and dominant form of urease found in the environment where there are large amounts of urea present. The poultry litter which is the mixture of different things contain huge amounts of ureolytic bacteria. The urease producing bacteria in poultry liter includes *alcaligenes*, *aeromonas*, *bacillus*, *corynebacterium*, and *micrococcus*. There are several methods used to reduce ammonia emission in poultry sheds. To reduce ammonia concentration from the sheds heat exchange air cleaning system is being use. There is adjustable nozzle that allows the inlet air into the shed. In this way, the air is distributed within the shed through adjustable nozzle and airflow that is produced by gas heaters and circulation fans. Chemical method includes the chemicals like sodium bisulfate. It converts the litter ammonium, to ammonium sulfate and reduces the pH of litter. Ammonium sulfate is a water-soluble fertilizer. Another important source to reduce ammonia in poultry sheds is the medicinal plants and they have good antimicrobial activity. A phytogetic additive plays a significant role to increase food digestion in poultry and reduce inflammation and antioxidant activity in broiler, one of phytogetic feed additives digestrom regulates the transcription factor nrf2 to reduce inflammation. Plants are being use as alternative source instead of antibiotics and plays significant role in ammonia emission control in poultry sheds. The plant which has good activity against urease production is yucca plants extract. The *aloeavera* gel and leaf has good activity against urease producing bacteria and plays significant role in ammonia emission.

Review of Literature

Background

Ammonia formation occurs in poultry litter due to microbial activities, some microbes produce urease enzyme, which degrades the uric acid in poultry litter and convert uric acid into ammonia. The litter contains gram positive bacteria as well as gram negative bacteria, like *Staphylococcus*, *Bacillus*, *Aeromonas* and *Escherichia coli*.

Broiler litter microbiology and gut microbes

The poultry litter has different kinds of microorganisms. The litter contains vast amounts of microorganisms up to 10¹⁰ to 10¹¹ colony forming unit per gram of litter. Different types of bacteria found in broiler litter like *Escherichia coli*, *Clostridium perforenges*, *Staphylococcus*, *Campylobacter* and *Salmonella*. *Escherichia coli* and *Salmonella* are gram negative bacteria while *Staphylococcus* and *Clostridium* are gram positive bacteria. The *Escherichia coli* are gram negative rods non motile or motile and ferment lactose, and act normal flora in poultry intestine. *Escherichia coli* are anaerobic and act as opportunistic pathogen and cause urinary tracts or food burn disease in human.

Campylobacter is gram negative bacterium spiral rod shape and shows motility by containing a single polar flagellum, present in poultry litter also present in broiler gut. They are the major pathogen of human of poultry meat origin and cause food burn illness such as Campylobacteriosis. *Clostridium perforenges* are gram positive spore forming bacterium present in broiler litter and cause gas gangers in human. They are food burn pathogen and cause food burn disease rather than human health. They also cause problem in broiler. *Clostridium perferenges* cause necrotic enteritis which may lead to mortality and morbidity. They also cause gangrenous dermatitis which is associated with older broiler and can cause certain mortality. *Salmonella* is gram negative bacteria rod shape, motile, facultative anaerobes and H₂S producer. They are the causative agents of septicemia, typhoid, and gastroenteritis's. *Salmonella* are the leading pathogen of human food burn diseases approximately more than 1 million cases reported per year in the United States. *Staphylococcus* is gram positive bacteria, cocci non motile, catalase positive and cause skin infection in human. They act as normal flora on human skin. They are present in poultry litter in highest percentage than other bacteria. *Staphylococcus* produces

different types of toxin and cause several infections in human. They cause food illness problem in human.

Urease producing bacteria in broiler

The urease-producing bacteria in poultry litter are *Proteus mirabilis*, *Helicobacter pylori*, *Aeromonas*, *Bacillus*. *Helicobacter pylori* are a spiral shaped bacterium that causes ulcer in human. *Helicobacter pylori* cause more than 90% of duodenum ulcer and up to 80% gastric ulcer in human. They are present in poultry litter and urease positive bacteria responsible for ammonia emission in poultry litter. *Proteus mirabilis* are gram negative facultative anaerobic and rod shaped bacteria. They are also the urease producing bacteria in broiler litter.

Urease enzyme

Bacterial urease is present in considerable amount in environment. Urease enzyme plays significant role in urea and uric acid conversion into ammonia. There are so many forms of urease enzyme but most important is trimeric structure that is conserved in all. Nearly all bacteria urease have heteropolymeric mean having different shapes and structure. The four bacterial enzymes which are mostly studied are *Proteus mirabilis*, *Bacillus subtilis*, *Klebsiella aerogenes* and *Helicobacter pylori*. Urease have three different subunits UreA, UreB and UreC. Urease enzyme function or activity mostly dependent on the level of intracellular Ni²⁺ ion, if concentration of ions is in greater amount than urease exhibit the toxic effects.

Ammonia dematerialization from broiler

Ammonia dematerialization from poultry occur at all stages of litter handling. Huge amount of ammonia loss occur during broiler facilities and land application. Patterson showed that 62% of ammonia emission occurs from poultry litter during housing. A different plant extracts uses as feed additives and other method to reduce ammonia emission in poultry farms and shades. Other method include the ventilation system, is uses to reduce ammonia emission. This is not good method regarding energy cost concern and environmental problem.

Effects of ammonia in broiler

Ammonia is dangerous gas that create respiratory tract problem in poultry by destroying poultry cilia. One hundred parts per volume of ammonia caused reductions in the respiration of mature

chicken range between seven to twenty-four. There are number of methods and techniques used to reduce the wasted nitrogen level especially ammonia in broiler. Ammonia has important effects on broiler health especially on their eyes. If the level of ammonia increase from 50-100ppm than ammonia dissolve in the eyes. This ammonia reacts with water and formation of ammonium hydroxide occurs. Ammonium hydroxide is also toxic and cause Keratoconjunctivitis. If the level of the ammonia increases than 100ppm, it may cause blindness and corneal ulceration. According to if the concentration of ammonia increases from normal level in poultry sheds, it leads to different bacterial infection occur like *E. coli* infection the most common infection. Increase levels of ammonia have also negative effects on bird's body weight and most probably affect immune system of broiler. Cilia are tiny hair like projection present on epithelial cell. High level of ammonia in poultry sheds may lead to the paralysis of cilia, due to which loss of epithelial cell occur. When cilia become damage so most of bacteria attached to the cilia. When cilia damage a lot of lower respiratory tract problems occur in birds.

Statement of problem

Ammonia is one of the toxic gas releases in poultry sheds. The poultry manure contain Urease producing bacteria that produce Urease enzyme, which break down the urea, or uric acid due to which ammonia will form. During humidity, ammonia react with moisture due to which ammonium ions (NH₄⁺) form. This ammonium is toxic for broiler, which disrupt the cilia of broiler. It leads to most of bacterial infections. It can also cause paralysis and Keratoconjunctivitis in broiler. The use of medicinal plants extract can be effective way to control ammonia emission in poultry sheds and help to reduce the level of Urease producing bacteria in broiler.

Hypothesis

Medicinal plant extracts mitigate ammonia emission from poultry gut/droppings by altering the gut microbiota and arresting urease enzyme activity.

Objectives

- To determine effect of plant extracts on gut microbial flora of broiler
- To determine the effect of medicinal plant effect on ammonia emission from poultry/poultry litter.

Materials and Methods

Medicinal plants

Some medicinal Plants will be selected including i.e., *Allium sativum* (Garlic), *Piper nigrum* (Black pepper), *Cuminumcyminum* (Safaidzeera), *Aloe Barbadensis* (Aloe vera) and *Yucca schidigera* will be collected from different natural resources or their extract can be procured from commercial market and used.

Medicinal plant extracts preparation

From above mentioned medicinal plants, aqueous and alcoholic extract will be prepared by drying the leaves of selected plants and then dry leaves dissolve properly in distilled water or 95% ethanol. This dissolved solution will be incubated at room temperature with continuous shaking for the period of 3 days. After Incubation, filtration of the extract will be done by using Whatman filter paper no.2.

Activity of plant extract against urease positive bacteria

Medicinal plants extracts will be used against urease positive bacteria. Urease positive bacteria will be collected by procuring the samples from poultry farms and isolated by different bacterial culturing methods. Antibacterial will be formed through well diffusion assay.

In vitro effect of plant extracts on ammonia emission from poultry droppings

Effect of all plant extracts on ammonia emission from intestinal contents/droppings *in vitro* will be determined as describe by with minor modifications. Briefly, 100g poultry droppings will be mixed with 0.1g of plant extract and incubated at 37c for 24h and emitted ammonia will be measured. One setup of poultry dropping alone will serve as control. Urease enzyme activity of each setup will also be measured.

In vivo effect of plant extracts on ammonia emission from poultry droppings

In vivo effect of selected plant extracts on ammonia emission from broiler droppings will be determined by following the method describe previously. Briefly, day old broiler (n = 50) will be purchased from local market and reared in poultry shed. Birds will be divided randomly into 05 groups (50 birds in each group). One group will serve as negative control and receive no plant

extract. Three groups will receive two selected plant extracts and *Yucca* extract, respectively from day 16 to 25. Group (05) will serve as positive control and receive a commercial ammonia inhibitor sodium bisulfate. Droppings of each group will be collected on alternate days and ammonia emission and urease activity will be determined.

Effect of plant extracts on gut microbiota

Birds will be slaughtered at day 26 and their intestinal contents will be collected and pooled. DNAs from pooled sample of each group will be extracted using commercial DNA kit following the manufacturer's instructions and gut microbial diversity will be determined by molecular methods/Next Generation Sequencing.